植物研究雑誌
J. Jpn. Bot.
73: 128–136 (1998)

Cytogenetic Studies on Wild *Chrysanthemum* sensu lato in China VI. Karyomorphological Characters of Five Species of *Ajania* and Each One Species of *Brachanthemum, Dendranthema, Elachanthemum, Phaeostigma* and *Tanacetum* in Highlands of Gansu, Qinghai and Sichuan Provinces

Katsuhiko KONDO[a], Ryuso TANAKA[a], Masahiro HIZUME[b], Goro KOKUBUGATA[a,1)], Deyuan HONG[c], Song GE[c] and Qiner YANG[c]

[a]Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University, Kagamiyama 1-4-3, Higashi-Hiroshima, 739-8526 JAPAN;
[b]Biological Institute, Faculty of Education, Ehime University, Matsuyama, 790-0826 JAPAN;
[c]Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Peking, 100093 CHINA

(Received on September 8, 1997)

Ten species of *Chrysanthemum* in broad sense or of six genera in narrow sense in the Artemisiinae, the Anthemideae, the Compositae, collected in highlands in Gansu, Qinghai and Sichuan Provinces, China, were chromosomally studied. The interphase chromosomes of complex chromocenter type, prophase chromosomes of interstitial type and basic chromosome number of x=9 were common to all the species examined, but the karyotypes showed some intergeneric differences.

(Continued from J. Jpn. Bot. **70**: 85–94, 1995)

Among the members of *Chrysanthemum* in broad sense taxonomically placed in the tribe Anthemideae Cass. (Compositae) (Bremer and Humphries 1993), *Achillea* L., *Ajania* Poljakov, *Brachanthemum* DC., *Dendranthema* (DC.) Des Moul., *Elachanthemum* Ling & Y. R. Ling, and *Tanacetum* L., all in narrow sense, can be seen in highlands of Gansu, Qinghai and Sichuan Provinces, China. They could be biosystematically and genetically closely related to each other because some combinations among them hybridize in nature or under artificial conditions (e.g., Tanaka and Shimotomai 1978). Additionally, the monotypic *Elachanthemum* previously placed in *Artemisia* (Ling and Ling 1978) is considered to be a bridge between *Ajania* and *Artemisia* L. (Yang unpublished). Thus, they should be chromosomally examined to clarify their cytotaxonomic relationships.

Extensive chromosome investigations on wild *Chrysanthemum* in broad sense in China began in 1989 in a Japanese-Chinese joint research (e.g., Nakata et al. 1991a, 1991b, Tanaka 1992). Members of *Chrysanthemum* in broad sense always showed the basic chromosome number of x=9 (e.g., Kondo et al. 1992, Kondo and Tanaka 1996). This paper is the sixth of our research series on Chinese *Chrysanthemum* in broad sense.

Materials and Methods

The plants and seeds of *Ajania fruticulosa* (Ledeb.) Poljakov, *A. khartensis* (Dunn) Shih, *A. latifolia* Shih, *A. myriantha* (Franch.) Ling ex Shih, *A. nematoloba* (Hand.-Mazz.) Ling & Shih, *Brachanthemum pulvinatum* (Hand.-Mazz.) Shih, *Dendranthema glabriusculum* (W.W. Smith) Shih, *Elachanthemum intricatum* (Franch.) Ling & Y.R. Ling, *Phaeostigma salicifolium* (Mattf.) Muld. and *Tanacetum falcatolobatum* Krasch studied were collected in highlands of Gansu, Qinghai and Sichuan Provinces, China (Table 1). They were cultivated in pots in the conservation garden of the Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University. Voucher specimens are deposited at the Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University.

Root tips were harvested and pretreated in 0.002M 8-hydroxyquinoline at 18°C for 1.5 hours. They were fixed in 45% acetic acid at 4°C for 15 minutes, following which they were hydrolyzed in a 2:1 mixture of 1N-HCl and 45% acetic acid at 60°C for 12 seconds, and squashed in 1% aceto-orcein.

Karyomorphological classification of interphase and mitotic prophase chromosomes followed Tanaka (1971). The karyotype formula of each species was based on chromosome characters of ten somatic metaphase cells. The position of the centromere of chromosome followed Levan et al. (1964): Arm ratio was calculated by long arm/short arm. Values of 1.0–1.7 were grouped as chromosomes with median centromeres; 1.8–3.0 submedian centromeres; 3.1–7.0 subterminal centromeres; and 7.1 or more terminal centromeres.

Results and Discussion

Common characteristics of karyomorphology were observed in the chromosomes at interphase and mitotic prophase, respectively, in all the genera of *Chrysanthemum* in broad sense studied here (Figs. 1 and 2) and elsewhere (Kondo et al. 1992, 1995, Kondo and Tanaka 1996): The interphase nucleus was of the complex chromocenter type and the mitotic prophase chromosomes was of the interstitial type, which was characterized by the chromocenters, i.e. the heterochromatic segments located in the proximal region as well as in the interstitial and distal regions of most of the chromosomes of the complement (Figs. 1 and 2).

The chromosome numbers and karyotypes in ten highland species of *Chrysanthemum* in broad sense or six genera in narrow sense in the Chinese Anthemideae are shown in Figs. 3 and 4. No microphotographic figure of chromosomes of *Ajania nematoloba* was shown because its highest chromosome number of

Table 1. Localities, identification numbers and chromosome numbers of some highland species of *Chrysanthemum* in broad sense from Gansu, Qinghai and Sichuan Provinces, China

Species	I.D. No.	Chromosome No. (2n)	Locality
Ajania fruticulosa (Ledeb.) Poljakov	10-8-1	36	Qinghai Province: Xunhua Co., Xiatanzhuang, Huanghe Valley alt. 1,720 m
	10-10-3	36	Qinghai Province: Tongren Co., Bao'anxiang Xiegou, alt. 2,300 m
	10-12-2	36	Qinghai Province: Jianzha Co., Zhiganglaka, Xialijia, alt. 2,100 m

A. khartensis (Dunn) Shih	10-11-2	54	Qinghai Province: Tongren Co., Guashize, alt. 3,500 m
A. latifolia Shih	15	36	Gansu Province: Wen Co., Caopingliang, alt. 2,150 m
A. myriantha (Franch.) Ling ex Shih	10-7-1	18	Qinghai Province: Pin'an Co., Guchenxiang shibi, alt. 2,840 m
	10-7-3	18	Qinghai Province: Hualong Co., Gandu Valley, alt. 2,550 m
	10-8-2	18	Qinghai Province: Xunhua Co., Dongshaogon, Huanghe Valley, alt. 1,710 m
	10-8-3	18	Qinghai Province: Xunhua Co., Mengda, Tianci
	10-11-1	18	Qinghai Province: Tongren Co., Shuangpengxi Forest, alt. 2,950 m
A. nematoloba (Hand.-Mazz.) Ling & Shih	10-8-1	72	Qinghai Province: Xunhua Co., Xiatanzhuang, Huanhe Valley, alt. 1,720 m
Brachanthemum pulvinatum (Hand.-Mazz.) Shih	10-8-1	18	Qinghai Province: Xunhua Co., Xiatanzhuang, Huanghe Valley, alt. 1,720 m
Dendranthema glabriusculum (W.W. Smith) Shih	26	54	Sichuan Province: Zhaojue Co., alt. 1,950 m
Elachanthemum intricatum (Franch.) Ling et Y.R. Ling	10-12-1	18	Qinghai Province: Jianzha Co., Kanbula, alt. 2,150 m
Phaeostigma salicifolium (Mattf.) Muld.	10-9-1	18	Qinghai Province: Xunhua Co., Mt. Dalijiashan, alt. 2,920 m
	10-10-2	18	Qinghai Province: Xunhua Co., Gangcagou, alt. 2,650 m
	10-11-1	18	Qinghai Province: Tongren Co., Shuang-pengxi Forest, alt. 2,950 m
Tanacetum falcatolobatum Krasch	10-7-4	18	Qinghai Province: Hua Long Co., Gandu Valley, alt. 2,350 m
	10-10-3	18	Qinghai Province: Tongren Co., Bao'anxiang Xiegou, alt. 2,300 m
	10-12-2	18	Qinghai Province: Jianzha Co., Zhiganglaka, Xialijia, alt. 2,100 m

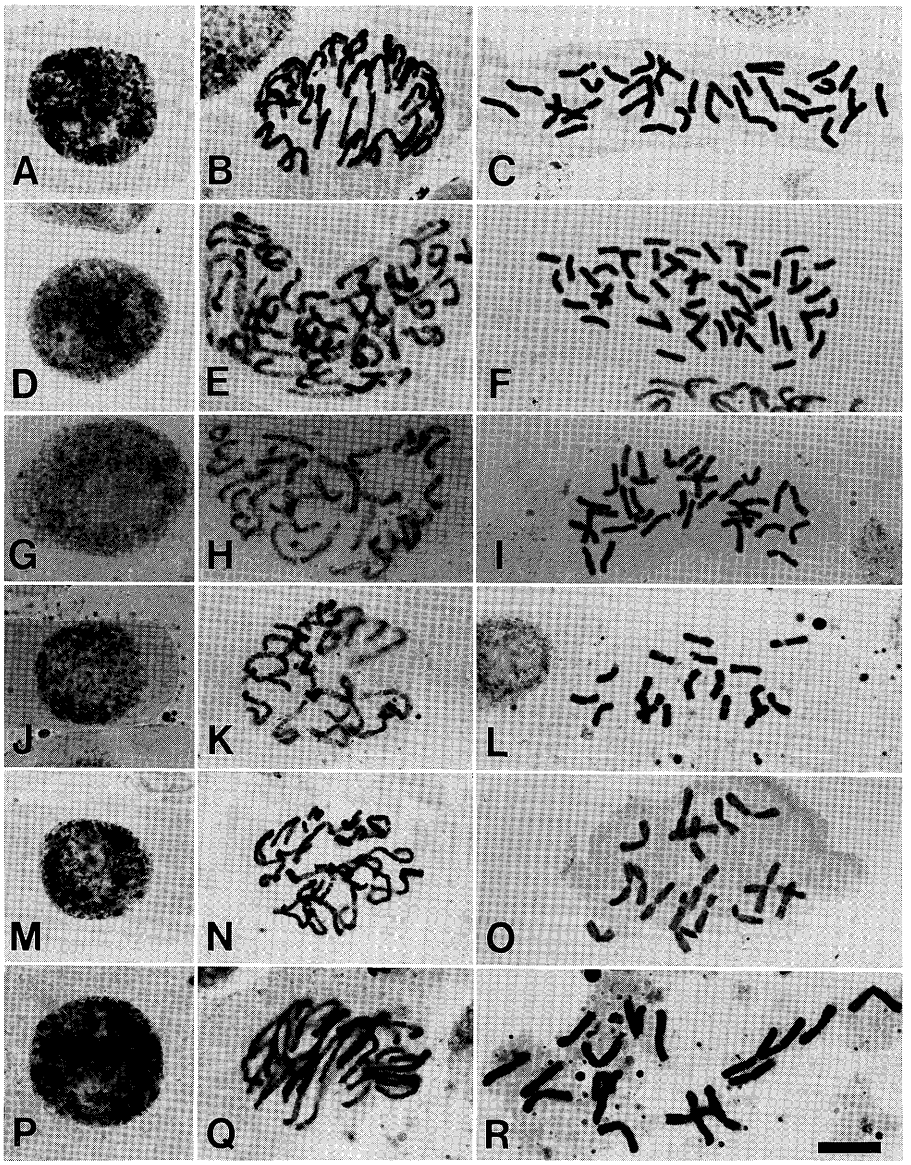

Fig. 1. Karyomorphology of six species of *Chrysanthemum* in broad sense. A–C. *Ajania fruticulosa.* D–F. *Ajania khartensis.* G–I. *Ajania latifolia.* J–L. *Ajania myriantha.* M–O. *Brachanthemum pulvinatum.* P–R. *Tanacetum falcatolobatum.* A, D, G, J, M and P. Interphase nuclei. B, E, H, K, N and Q. Mitotic prophase chromosomes. C, F, I, L, O and R. Mitotic metaphase chromosomes. Bar = 10 μm.

2n=72 and large chromosome sizes disturbed preparations. The chromosome numbers and karyotypes of *Ajania khartensis* (2n=54, Figs. 1F and 3B), *A. latifolia* (2n=36, Figs. 1I and 3C), *A. nematoloba* (2n=72), *Brachanthemum pulvinatum* (2n=18, Figs. 1O and 3E), *Dendranthema glabriusculum* (2n=54, Figs. 2C and 4A) and *Elachanthemum intricatum* (2n=18, Figs. 2I and 4B) and their karyotypes were reported here for the first time. The

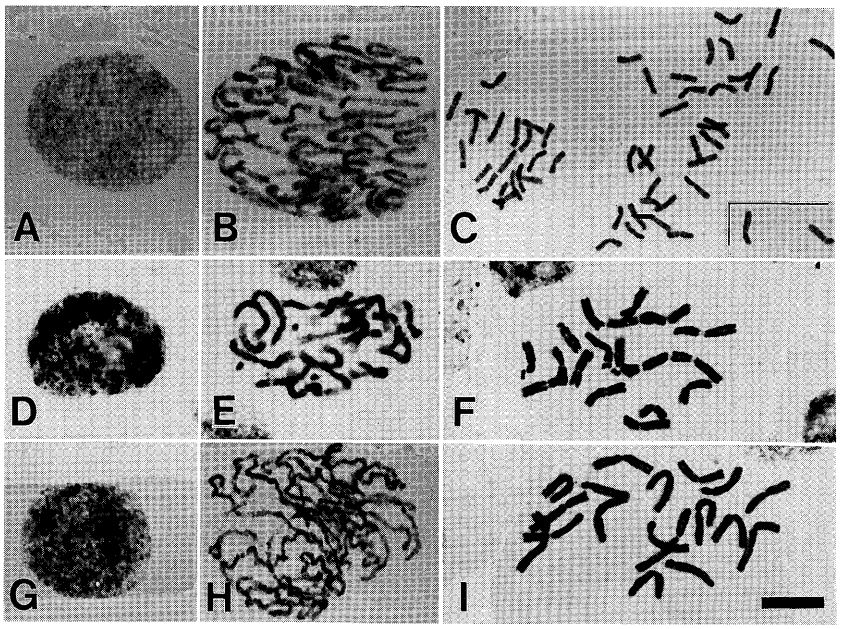

Fig. 2. Karyomorphology of three species of *Chrysanthemum* in broad sense. A–C. *Dendranthema glabriusculum.* D–F. *Elachanthemum intricatum.* G–I. *Phaeostigma salicifolium.* A, D and G. Interphase nuclei. B, E and H. Mitotic prophase chromosomes. C, F and I. Mitotic metaphase chromosomes. Bar=10 μm.

karyotypes of *Ajania fruticulosa*, *A. myriantha*, *Phaeostigma salicifolium* (formerly *Ajania salicifolia* (Mattf.) Poljakov), and *Tanacetum falcatolobatum* (formerly *Cancrinia maximowiczii* C. Winkl.) have already been described (Kondo et al. 1995, Kondo and Tanaka 1996).

The plants of *Ajania fruticulosa* studied here had a karyotype with 2n=36 (Fig. 1A), which was different from the diploid karyotype with 2n=18 in the plants collected in Lanzhou City, Gansu Province (I.D. No. 92-38-1) (Kondo and Tanaka 1996). The Qinghai plants 10–20 cm tall were smaller than the Lanzhou plants 20–40 cm tall. The former plants propagate rapidly by adventitious budding on roots while the latter plants propagate rapidly by rooting from the nodes of the stems touching the ground. It has not yet been clarified whether the karyotype with 2n=36 in *A. fruticulosa* was originated from the diploid karyotype of the species with 2n=18 by simple chromosome doubling or by hybridizing.

Ajania myriantha in Qinghai populations showed only diploid (2n=18) (Fig. 1D). This species has been reported previously to have two cytotypes with 2n=18 (diploid) and 36 (tetraploid) (Kondo and Tanaka 1996).

Ajania khartensis (hexaploid, 2n=54) was very similar in creeping growth habit to *A. tenuifolia* (tetraploid, 2n=36, Kondo et al. 1992) but different in chromosome number.

Ajania fruticulosa had the karyotype of 2n=36=16m+8sm+1sm^{sat}+8st+3st^{sat}, *A. khartensis* 2n=54=30m+1m^{sat}+9sm+8st+6st^{sat}, *A. latifolia* 2n=36=15m+10sm+7st+4st^{sat}, *A. myriantha* 2n=18=6m+8sm+2st+2st^{sat}, *Brachanthemum pulvinatum* 2n=18=12m+6sm, *Dendranthema glabriusculum* 2n=54=18m+28sm+2st+6st^{sat}, *Elachanthemum intricatum* 2n=18=8m+6sm+4st^{sat}, *Phaeostigma salicifolium* 2n=18=10m+4sm+

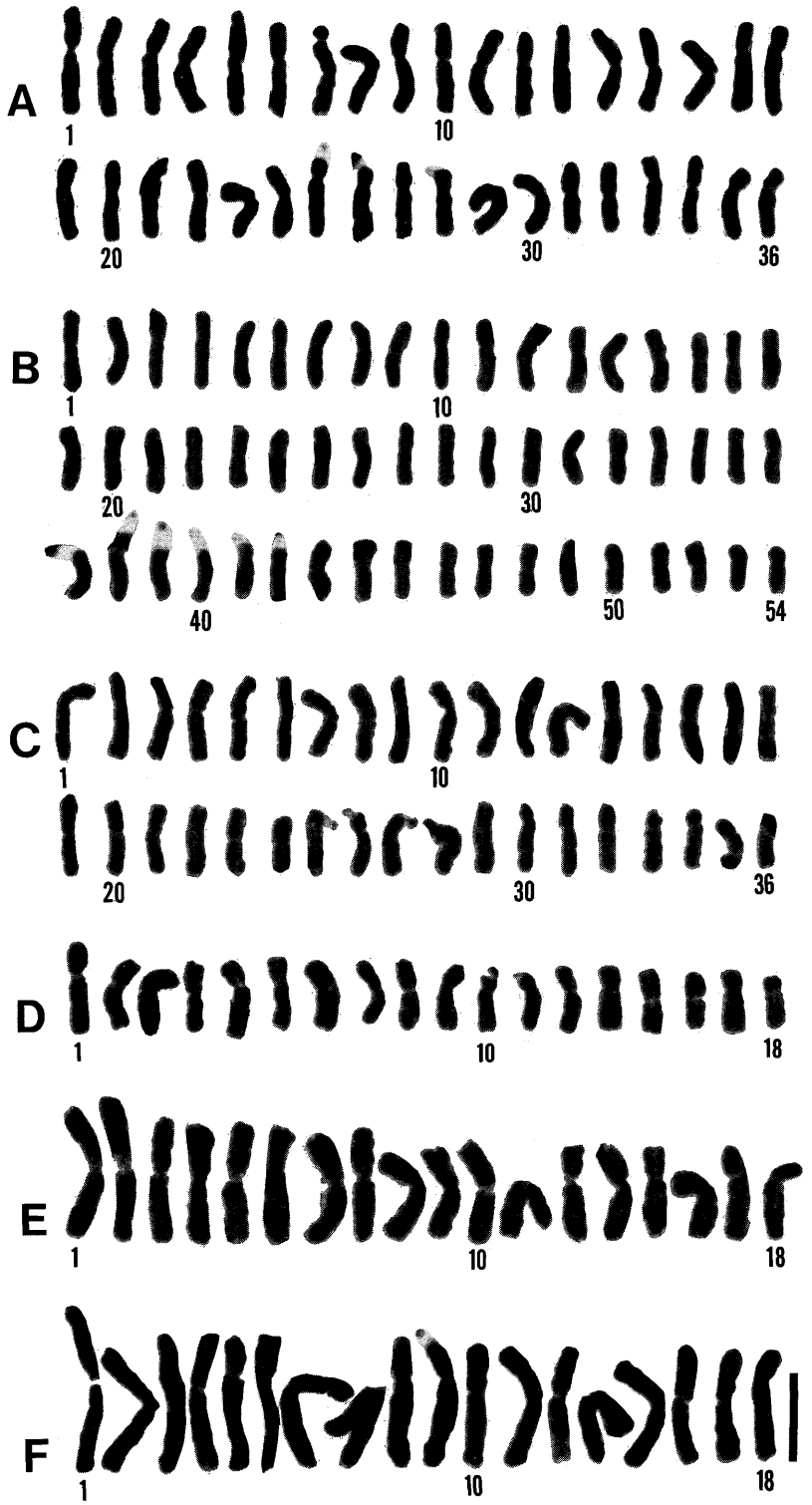

Fig. 3. Chromosome alignments in six species of *Chrysanthemum* in broad sense. A. *Ajania fruticulosa* (2n=36). B. *Ajania khartensis* (2n=54). C. *Ajania latifolia* (2n=36). D. *Ajania myriantha* (2n=18). E. *Brachanthemum pulvinatum* (2n=18). F. *Tanacetum falcatolobatum* (2n=18). Bar=10 μm.

1st+3st^{sat} and *Tanacetum falcatolobatum* 2n=18=13m+1sm+3st+1st^{sat} (Figs. 3 and 4). The karyotypes of the diploid plants of *Ajania* and *Phaeostigma* studied here agrees perfectly with Kondo and Tanaka's (1996) statement that the diploid plants of the two genera may show a common karyotype with 14 median- or submedian- and four subterminal-centromeric chromosomes. The karyotype of Qinghai plants of *Tanacetum falcatolobatum* (Fig. 3F) was exactly the same as the Gansu plants (Kondo et al. 1995, Kondo and Tanaka 1996). The karyotype of *Elachanthemum intricatum*, which was formerly treated as *Artemisia intricatum* Franch., also showed the same karyotype as the diploid plants of *Ajania* and *Phaeostigma*. Among the taxa studied here, *Brachanthemum pulvinatum* had more symmetric karyotype than the other genera (Fig. 3E) and *Phaeostigma salicifolium* had the karyotype with the largest chromosomes in length (Fig. 4C). Numbers of satellites visualized by orcein-staining here ranged from 0 to 4 in the diploid (2n=18) species, 4 in the tetraploid (2n=36) species, and from 6 to 7 in the hexaploid (2n=54) species (Figs. 3 and 4), although exact counts of satellites in *Chrysanthemum* in broad sense should be made by our technique (Kondo et al. 1996) of the fluorescence in situ hybridization (FISH).

Fig. 4. Chromosome alignments in three species of *Chrysanthemum* in broad sense. A. *Dendranthema glabriusculum* (2n=54). B. *Elachanthemum intricatum* (2n=18). C. *Phaeostigma salicifolium* (2n=18). Bar=10 μm.

Karyomorphological similarities in interphase and mitotic prophase chromosomes, the same basic chromosome number of X=9 and karyotypic similarities especially among the diploid taxa in *Chrysanthemum* in broad sense in the tribe Anthemideae studied here suggested that they would be closely related and could be placed in the same genus. Further experiments such as interspecific and intergeneric crossability, FISH and genomic in situ hybridization (GISH) of the chromosomes, should be expected to clarify relationships among the species and genera in *Chrysanthemum* in broad sense.

This project was funded in part by the Monbusho International Scientific Research Program: Joint Research (No. 06044160; Representative: K. Kondo) of the Japanese Ministry of Education, Science, Sports and Culture for 1994–1996. This paper was contributed from Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University (Contribution No. 54).

Endnote

[1)]Present address: Tsukuba Botanical Garden, National Science Museum, Amakubo 4-1-1, Tsukuba, 305-0005 JAPAN.

References

Bremer K. and Humphries C. J. 1993. The generic monograph of the Asteraceae-Anthemideae. Bull. Nat. Hist. Mus. London (Bot.) **23**: 71–177.

Kondo K., Honda Y. and Tanaka R. 1996. Chromosome marking in *Dendranthema japonicum* var. *wakasaense* and its closely related species by fluorescence in situ hybridization using rDNA probe pTa71. La Kromosomo **II-81**: 2785–2791.

——— and Tanaka R. 1996. Chromosome studies on *Ajania* and its allied genera. *In*: D. J. N. Hind and H. J. Beentje (eds.), Compositae: Systematics. Proceedings of the International Compositae Conference, Kew, 1994. **I**: 425–434. Royal Botanic Gardens, Kew.

———, Tanaka R., Ge S., Hong D. and Nakata M. 1992. Cytogenetic studies on wild *Chrysanthemum sensu lato* in China. IV. Karyomorphological characteristics of three species of *Ajania*. J. Jpn. Bot. **67**: 324–329.

———, Tanaka R., Hong D., Hizume M., Yang Q. and Nakata M. 1995. Cytogenetic studies on wild *Chrysanthemum sensu lato* in China V. A chromosome study of three species of *Ajania*, *Cancrinia maximowiczii* and *Dendranthema lavandulifolium* in the Chrysantheminae, the Anthemideae, the Compositae in Chinese highlands. J. Jpn. Bot. **70**: 85–94.

Levan A., Fredga K. and Sandberg A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.

Ling Y. and Ling Y. 1978. *Elachanthemum*, genus novum familiae Compositarum. Acta Phytotax. Sinica **16**: 61–65 (*in Chinese*).

Nakata M., Hong D., Liu D., Hoshino T., Zhong Z., Tanaka R. and Chen R. 1991a. Cytogenetic studies on wild *Chrysanthemum sensu lato* in China. III. Karyotype of *Dendranthema oreastrum*. Chromosome Information Service **51**: 16–18.

———, ———, Qiu J., Uchiyama H., Tanaka R. and Chen S. 1991b. Cytogenetic studies on wild *Chrysanthemum sensu lato* in China. I. Karyotype of *Dendranthema vestitum*. J. Jpn. Bot. **66**: 199–204.

Tanaka R. 1971. Types of resting nuclei of Orchidaceae. Bot. Mag. Tokyo **84**: 118–122.

——— (ed.) 1992. Cytogenetics on plants correlating between Japan and China. Report of 1988–1991 Grant-in-Aid for Monbusho International Scientific Research Program: Joint Research (Nos. 63044176–01044099), Hiroshima University.

近藤勝彦[a]，田中隆荘[a]，日詰雅博[b]，國府方吾郎[a,1)]，洪　徳元[c]，葛　頌[b]，楊　親二[c]：**中国産野生キク属（広義）の細胞遺伝学的研究　VI. 甘粛，青海，四川各省高地に分布する Ajania 属 5 種および Brachanthemum, Dendranthema, Elachanthemum, Phaeostigma, Tanacetum 各属1種の核形態学的特徴**

中国甘粛，青海，四川各省高地産*Ajania*属5種および*Brachanthemum*，*Dendranthema*，*Elachanthemum*，*Phaeostigma*，*Tanacetum*各属1種は，共通して複雑染色中央粒型間期染色体，介在型分裂期前期染色体の形態を示した．*Ajania khartensis*（2n=54=30m+1m[sat]+9sm+8st+6st[sat]），*A.*

latifolia (2n=36=15m+10sm+7st+4st^{sat}), *A. nematoloba* (2n=72, 核型未決定), *Brachanthemum pulvinatum* (2n=18=12m+6sm), *Dendranthema glabriusculum* (2n=54=18m+28sm+2st+6st^{sat}), *Elachanthemum intricatum* (2n=18=8m+6sm+4st^{sat}) の染色体数と核型は本論文が初めての発表である．*Ajania fruticulosa*は，すでに染色体数2n=18とその核型が発表されているが，新たに染色体数と核型2n=36=16m+8sm+1sm^{sat}+8st+3st^{sat}を報告した．

([a]広島大学理学部附属植物遺伝子保管実験施設, [1)]国立科学博物館筑波実験植物園, [b]愛媛大学教育学部生物学教室, [c]中国科学院植物研究所)